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Interaction between Cadmium and Selenium in Rat Plasma

by T. A. Gasiewicz*† and J. C. Smith*

The metabolism of ^{75}Se-labeled SeO_3^{2-} and its conversion by intact rat erythrocytes *in vitro* to a form which complexes with Cd and plasma proteins were studied. By utilizing both excess SeO_3^{2-} and N-ethylmaleimide to lower erythrocyte reduced glutathione (GSH) concentrations, it was shown that the uptake and metabolism of SeO_3^{2-} were GSH-dependent, the probable intermediate being glutathione selenotrisulfide (GSSeSG). Secondary release of selenium by rat erythrocytes had no relation to the erythrocyte transport of oxidized glutathione (GSSG). While fluoride depressed and chromate increased GSSG transport, chromate, a glutathione reductase inhibitor, decreased selenium release. This release appeared to be secondary to a reaction catalyzed by glutathione reductase. The similarity of I_{50} values for chromate's inhibition of glutathione reductase and for the inhibition of selenium release further suggested a relationship between these two events. H_2Se or a similar product of GSSeSG reduction is proposed to be the active product of SeO_3^{2-} metabolism by rat erythrocytes. By use of gel-filtration and ion-exchange methods it was noted that the incubation of H_2Se with cadmium and plasma produced a Cd–Se complex indistinguishable from that produced by incubation of Cd, SeO_3^{2-}, plasma, and erythrocytes *in vitro*, or that noted following the administration of Cd and SeO_3 *in vivo*. A mechanism whereby the tissue distribution and toxicity of cadmium are altered by selenium is suggested.

Introduction

Following the observation of Kar, Das, and Mukerji (*1*) that the testicular lesions produced by cadmium could be prevented by the administration of selenium compounds, a number of workers have demonstrated the protective action of selenium compounds, especially of selenite (SeO_3^{2-}), on many of the biological effects of cadmium. The reversal of selenium toxicity by cadmium has also been demonstrated (*2*).

Although selenium completely protected against cadmium-induced testicular damage in rats, the uptake of cadmium by testicular tissue was increased (*3*). This suggested that selenium diverted cadmium from its usual target in the testes. Cadmium concentrations in other tissues were also altered, but the most pronounced change was in plasma, which showed a 30- to 50-fold increase in cadmium levels over control (*4, 5*). The distribution of cadmium in the soluble proteins of tissues was also altered by selenium (*6, 7*), and a Cd–Se-containing moiety appeared which moved at the void volume of G-75. The Cd–Se-containing fractions of plasma were of higher molecular weight. Some preliminary data on the interaction of cadmium and selenium have been presented previously by the present authors (*8*). This paper provides more details on these interactions.

Cadmium and selenium given to rats simultaneously increased the plasma levels of both over the levels seen when each was given alone. The peak level is seen to be attained at 4 hr. The cadmium and selenium are bound to plasma proteins with an atomic ratio of approximately 1. Initially two peaks of 130,000 daltons and 330,000 daltons are seen. The lower molecular weight peak is at a maximum at 1 hr and declines thereafter with the concomitant appearance of a 420,000 dalton peak. The latter peak is at a maximum at 12 hr. The 300,000 dalton peak appears to persist for the 24-hr time period studied.

* Environmental Health Sciences Center and Department of Pharmacology and Toxicology, University of Rochester Medical Center, Rochester, New York 14642.

† Present Address: Toxicology Center, Department of Biochemistry, Vanderbilt University, Nashville, Tennessee 37203.

Results and Discussion

The Cd–Se complex did not form when Cd and SeO_3^{2-} were incubated with plasma *in vitro*. Upon incubation of cadmium and selenium with plasma and washed red cells, the plasma profile showed a single peak containing equimolar amounts of cadmium and selenium and a molecular weight of 130,000.

When SeO_3^{2-} was incubated with plasma and red cells, selenium was very rapidly taken up by the red cells and then released over the course of a few minutes into the plasma, presumably in the form of a metabolite. The time course of this process for a number of starting concentrations of selenium is shown in Figure 1. While the release is almost complete at low concentrations of SeO_3^{2-} (0.5 nmole/ml erythrocytes), this is not so at high concentrations (0.5 μmole/ml erythrocytes). One limiting factor for the release was the availability of binding sites in the plasma. Very little selenium was released when 2.5×10^{-7} mole of SeO_3^{2-} was incubated with 1 ml of red cells in phosphate-saline buffer, pH 7.4. The selenium released increased from 30% at equal volumes of red cells and plasma to 90% at a 7-fold excess of plasma over red cells.

The uptake, subsequent metabolism, and release of selenium by rat erythrocytes were dependent on the presence of reduced glutathione (Fig. 2). As the concentration of SeO_3^{2-} in the preparation was increased, there was a depletion of erythrocyte GSH, and the amount of Se released into the plasma decreased in parallel. However, at higher SeO_3^{2-} concentrations, above the concentration necessary for complete depletion of the red cell GSH, the proportion of selenium in the plasma rose. This plasma selenium was largely in the form of SeO_3^{2-}, showing

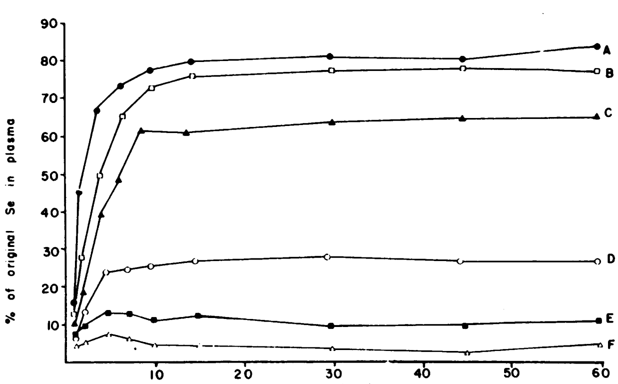

FIGURE 1. Kinetics of Se release by rat erythrocytes *in vitro* at initial SeO_3^{2-} concentrations of: (A) 5×10^{-10} mole; (*B*) 5×10^{-9} mole; (*C*) 5×10^{-8} mole; (*D*) 2.5×10^{-7} mole; (*E*) 5×10^{-7} mole; (*F*) 5×10^{-6} mole/ml of erythrocytes. Preparations of 0.2 ml washed rat erythrocytes plus 0.2 ml rat plasma (14*mM* glucose) were incubated for 15 min at 37°C prior to the addition of SeO_3^{2-}. At the appropriate times the exchange of selenium between erythrocytes and plasma was rapidly terminated by the addition of di-1-butylphthalate and immediate centrifugation at 1600*g* for 15 min.

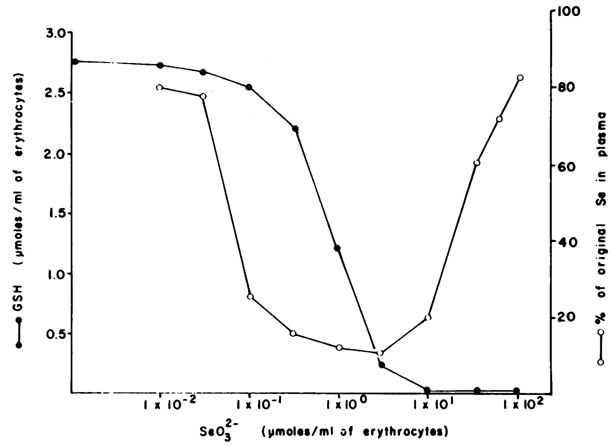

FIGURE 2. The depletion of erythrocyte GSH by increasing concentrations of SeO_3^{2-} and the corresponding distribution of Se at 30 min. Preparations consisted of 0.4 ml erythrocytes plus 0.4 ml plasma (14*mM* glucose). Incubation was for 15 min at 37°C prior to the addition of SeO_3^{2-}. After an additional 30 min incubation and centrifugation, 0.1 ml of packed erythrocytes was taken for GSH analysis. Each point is the mean of two determinations.

that metabolism had not taken place. Similar results were obtained when *N*-ethylmaleimide was used to deplete erythrocyte GSH. It seems likely that SeO_3^{2-} follows the reductive metabolism, as proposed by Ganther (*9*) and shown in Eq. (1)

$$H_2SeO_3 + 4SGH \rightarrow GSSeSG + GSSG + 3H_2O \qquad (1)$$

Sandholm (*10*) postulated that selenium was released from erythrocytes as a glutathione complex. Jenkins and Hidiroglou (*11*) suggested that most of the selenium released from bovine erythrocytes *in vitro* was in the form of GSSeSG. It was proposed that the mechanism of release was similar to the transport of GSSG.

Our conclusion is that the release of selenium is not related to the release of GSSG and that the form of selenium released is not GSSeSG.

Fluoride ion, an inhibitor of GSSG release, had no effect on selenium release from erythrocytes. However, chromate, an inhibitor of glutathione reductase (*12, 13*), while increasing the GSSG levels of both plasma and red cells, can bring about complete inhibition of selenium release from red cells (Fig. 3).

Within erythrocytes GSSG is normally maintained at very low levels by reduction by glutathione reductase and NADPH (*14*). Release of GSSG from erythrocytes could only be demonstrated when glucose or another source of reducing equivalents was withdrawn or glutathione reductase was inhibited by chromate (*13*). In cell-free preparations GSSeSG has been found to be a good substrate for yeast glutathione reductase (*15*), leading

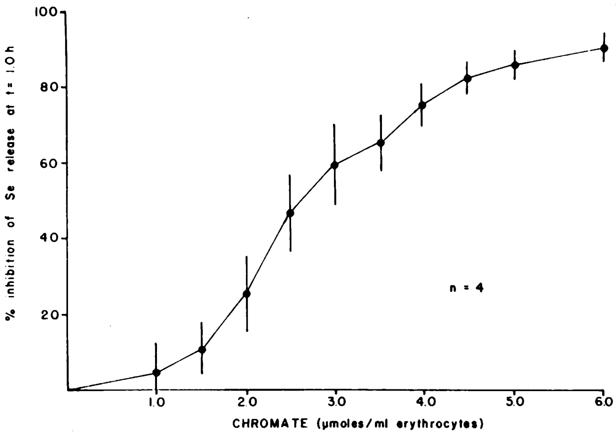

FIGURE 3. Release of Se from erythrocytes at 1 hr with various chromate concentrations. Washed erythrocytes (0.2 ml) plus 1.0 ml plasma (14*mM* glucose) were incubated 10 min at 37°C prior to the addition of the chromate. At 1 hr 1.0 ml of di-1-butylphthalate was added for the separation of erythrocytes and plasma. Each point represents the mean of four determinations; SD's are shown.

to the reduction of selenium to the −2 oxidation state as H_2Se or a similar selenide compound. It seems likely, therefore, that under physiological conditions GSSeSG would be further reduced by glutathione reductase. The similarity between the I_{50} values for chromate inhibition of glutathione reductase and inhibition of selenium release (0.35 and 0.25 μmole of chromate/pml of erythrocytes, respectively) may also suggest that selenium metabolism is linked to glutathione reductase.

As noted above, SeO_3^{2-} did not produce a complex with cadmium when incubated with plasma in the absence of erythrocytes. Neither did GSSeSG incubated with plasma. However, bubbling H_2Se [generated by the method of Diplock et al. (*16*)] produced a single Cd–Se peak with an atomic ratio 1 and a molecular weight 130,000. This peak is indistinguishable from that produced by incubating Cd, SeO_3^{2-}, plasma, and erythrocytes. It is also indistinguishable from the lower molecular weight peak seen in rat plasma following administration of Cd and SeO_3^{2-} *in vivo,* which is the predominant form of the Cd-Se complex shortly after administration.

If these three peaks were applied separately to QAE A-50 ion-exchange columns, each was eluted at the same portion of the gradient (Fig. 4). The Cd–Se complex was not associated with a major protein peak and had a charge density slightly less negative than albumin. The complex was apparently stable under the conditions of chromatography, which was carried out at pH 6.5.

When the G-150 gel chromatography was carried out at pH 6.5 instead of at pH 8 as previously, the Cd–Se complex formed by bubbling H_2Se into

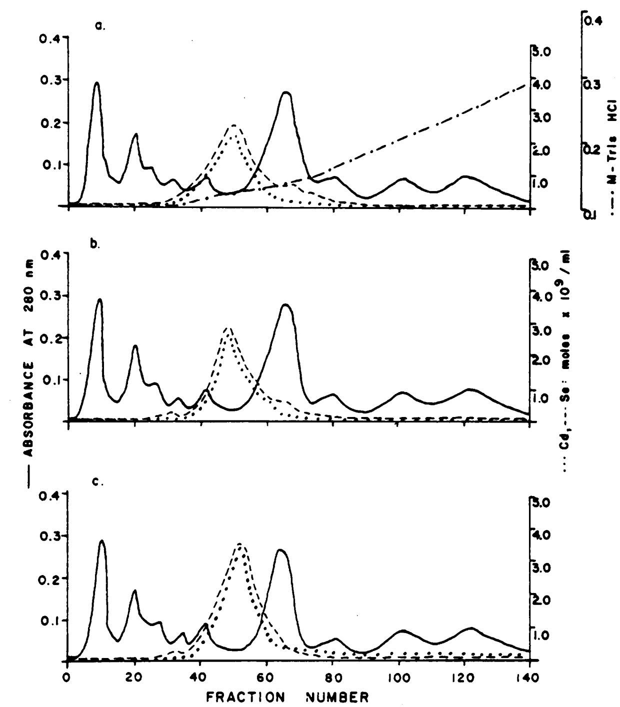

FIGURE 4. Sephadex QAE A-50 ion exchange chromatography of plasma: (*A*) 1.5 × 10^{-7} mole H_2Se bubbled into 2.0 ml plasma containing 1 × 10^{-7} mole Cd; (*B*) 1 × 10^{-7} mole SeO_3^{2-} added to a preparation containing 1.5 ml washed erythrocytes plus 1.6 ml plasma and 1 × 10^{-7} mole Cd, incubation for 1 hr at 37°C; (*C*) plasma from a rat injected subcutaneously with 20.0 μmole SeO_3^{2-}/kg and 20.0 μmole Cd/kg 1 hr before sacrifice. Ionic strength gradient shown same for (*A*), (*B*), and (*C*). The volume of whole plasma was 1.0 ml. Column dimensions were 1.6 × 24 cm. Starting buffer was 0.1*M* Tris–HCl, pH 6.5. Limiting buffer was 0.4*M* Tris–HCl, pH 6.5. Flow of buffer was 24.5 ml/hr. Fractions of 2.45 ml were collected.

plasma containing cadmium or by incubating SeO_3^{2-} with cadmium, plasma, and erythrocytes showed a molecular weight of 330,000. A similar pattern of Cd-Se distribution was found following the chromatography of plasma from rats injected with Cd and SeO_3^{2-} 1, 12, or 24 hr before sacrifice. These results were taken as evidence that the Cd–Se complex formed under these conditions *in vitro* was the same as formed *in vivo*. The metabolic path from SeO_3^{2-} to H_2Se has been documented by Ganther (*15*) and Hsieh and Ganther (*17*). This mechanism seems to exist both *in vitro* and *in vivo,* and seems to be the one whereby the tissue distribution of cadmium is altered and its toxicity reduced.

Only the 130,000 dalton form is produced by SeO_3^{2-} in the presence of erythrocytes and by H_2Se in their absence. The aggregation may be demonstrated by reducing the pH from 8.0 to 6.5 in gel chromatography.

Whether this or a similar complex is formed in compartments other than plasma is not known. If it is, then a large proportion of the cadmium dose may be sequestered in this manner. At 4-5 hr after simultaneous selenium and cadmium administration as much as 20% of the dose is in the form of the complex in plasma alone.

Because of the rapid metabolism of selenium *in vivo* and by erythrocytes *in vitro* the complex will only be formed if the selenium is not given too far ahead of the cadmium. The efficacy of SeO_3^{2-} in protecting against cadmium toxicity is much greater than that of SeO_4^{2-} and the seleno amino acids. Presumably these compounds must first be converted to SeO_3^{2-} or traverse an alternate pathway to H_2Se.

The fate of the Cd–Se complex has not been studied. The amount of the complex is about half its peak value at 24 hr. The excretion of cadmium is not significantly increased (*18*) and cadmium inhibits the urinary excretion of selenium, although six times as much selenium as cadmium appears in the excreta at 48 hr. The cadmium may deposit slowly in other tissues as the complex is decomposed. The liver burden of cadmium is less when selenium is given simultaneously so that other tissues must have higher levels. The slow rate of release of cadmium may allow secondary protection such as is afforded by the synthesis of metallothionein.

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